HABITAT USE OF PACIFIC HERRING (*CLUPEA PALLASII*) IN

PRINCE WILLIAM SOUND, ALASKA

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Dec 8, 2007

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# HABITAT USE OF PACIFIC HERRING (*CLUPEA PALLASII*) IN PRINCE WILLIAM SOUND, ALASKA

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

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Fairbanks, Alaska

December 2007

## Abstract

To determine the spawning area contributions of Pacific herring (*Clupea pallasii*) larvae to nursery bays, otolith chemical analysis was conducted on juvenile fish collected from 1995 to 1997 in Prince William Sound, Alaska. The otolith edge, representing the chemical signature of the known capture location, and the otolith core, representing the unknown spawning ground chemistry, were compared with discriminant function analysis to infer spawning area origin. Chemical signatures of $^{87}Sr/^{86}Sr$, $^{88}Sr/^{48}Ca$, $^{24}Mg/^{48}Ca$, and $^{138}Ba/^{48}Ca$ were used to identify broad spawning regions from inner and outer PWS that persisted for the three years sampling period despite significant interannual variability in otolith edge chemistry within nursery bays. Age of juvenile Pacific herring, age-0, 1, 2, did not significantly affect the otolith edge signatures; thus, this study is able to conclude from the otolith core chemistry that spawning areas do not contribute equally to nursery bays. This is the first demonstration that otolith chemical signatures can be used to identify the important spawning areas of this commercially important species in the Gulf of Alaska coastal areas.

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## Acknowledgements

My greatest gratitude is extended to my wife Anke, for her support and strength. I express my deepest appreciation to Dr. Brenda Norcross for serving as my committee chairperson, major professor, guide, for all of her support throughout this thesis research, and for her generosity in working with me long distance. I also extend my appreciation to the remaining members of my graduate committee Dr. Nate Bickford, Dr. Terrance Quinn II, and Dr. Brenda Konar for their helpful input into this project. I thank Steve Moffit from the Alaska Department of Fish and Game for collecting the adult herring samples. I thank the Oil Spill Recovery Institute and the Exxon Valdez Oil Spill Trustee Council for their generous fanatical assistance. I also thank Dr. Andrew Seitz for his superb revision contributions, an asset to my education and thesis work.

## Introduction:

Pacific herring (*Clupea pallasii* Valenceinnes 1847) is an ecologically, culturally, and economically important species. This forage fish is preyed upon by marine mammals, birds, invertebrates, and fish (Schweigert 1997). Pacific herring is an important subsistence resource for Native Alaskan users (Brown et al. 2002) and is commercially fished throughout its range from the Bering Sea to California (Mecklenburg et al. 2002). The commercial Pacific herring fishery in Prince William Sound (PWS), Alaska (Figure 1) began in the early 1900's (Brown et al. 2002) with an average annual ex-vessel value of $5.9 million from 1978 through 1988 (Ashe et al. 2005).

In March of 1989 the *Exxon Valdez* spilled crude oil into PWS during the Pacific herring spawning migration. The toxic effects of the oil spill on the PWS ecosystem caused the Pacific herring fishery to be closed shortly after the spill. Following the fishery closure, Pacific herring abundance continued to grow to a peak of 90,000 metric tons in 1992 (Ashe et al. 2005). The stock collapsed in 1993 due to the viral hemorrhagic septicemia virus (VHSV) and the fishery was closed 1994-1996 (Marty et al. 2003). There was limited commercial fishing for the following two years, but there has been no Pacific herring fishery in PWS since 1998 and the stock has not recovered to pre-oil spill abundance.

Pacific herring is a demersal spawner that moves into nearshore subtidal waters to deposit and fertilize its adhesive eggs (Hay 1990). Age-4 adult Pacific herring begin migrating from feeding areas to spawning areas in late March and

spawn in mid-April on 23–168 kilometers of coastline in PWS (Norcross et al. 2001). Spawning events occur in shallow coastal waters inside or outside of protected bays. Eggs are attached to macroalgae, eelgrass, and gravel or cobble substrate of the spawning ground; therefore, this area is the natal location of Pacific herring upon hatching. Attached eggs are lost to predation, wave-action, and exposure (Rooper et al. 1999). Surviving eggs incubate in these spawning areas for about 24 days before hatching as larvae in May (Brown et al. 1996). Larvae may be retained in or advected from the area, depending on larval behavior and local oceanographic conditions (Sinclair & Iles 1985). Larval Pacific herring metamorphose to the nekton in June of their hatch year (Stokesbury et al. 2002). In August, the young begin to form schools and aggregate at their nursery bay's heads (Stokesbury et al. 2000; Brown et al. 2002). These schools stay isolated in their respective bays until June of their second year when they leave the bays to join adult schools in coastal areas (Stokesbury et al. 2000).

Oceanic and atmospheric interactions influence the physical and chemical diversity of PWS Pacific herring natal and nursery areas. Located in northern Gulf of Alaska, PWS is a small inland sea (Muench & Heggie 1978) measuring 60 km wide and 90km long with depths exceeding 700 m. Elevation rises from sea level to 4000 m within 60 km of shore (Gay & Vaughan 2001). Orographic interactions between the Aleutian low and coastal mountains cause high levels of precipitation (Weingartner 2007), upwards of 5 m per year (Gay & Vaughan

2001). Numerous fjords, islands, and mountains support tidewater and alpine glaciers, which are seasonal sources of freshwater in PWS. The sound is connected to the Gulf of Alaska via Hinchinbrook Entrance and Montague Strait (Figure 1). Continental shelf water from the Gulf of Alaska (GOA) is driven by the Alaska Coastal Current (ACC) and Ekman transport to contribute water to PWS, especially from September to April (Niebauer et al. 1994; Vaughan et al. 2001). During this time, the flow in PWS is counterclockwise entering through Hinchinbrook Entrance and exiting through Montague Strait, and coastal downwelling occurs. During summer winds from the Aleutian low subside, Ekman transport is weakened, and there is deep-water inflow from the outer continental shelf. The deep-water inflow into PWS is a source of oceanic animals and nutrients in the region. The counterclockwise surface circulation can reverse as surface water enters through Montague Strait and exits through Hinchinbrook Entrance. During this time seasonal precipitation, weakened winds, and increased sunlight cause stratification and thus, highly productive conditions (Weingartner 2007).

Pacific herring's lifecycle and PWS's physical and chemical characteristics are conducive to the chemical imprinting of otoliths. Of the three pairs of calcified otolith structures found in the teleost auditory system, the sagittae is the largest and most studied (Wright et al. 2002). Otoliths are single cellular crystalline deposits of $CaCO_3$, in the form of aragonite, within an otolin-1 protein matrix. Otoliths, unlike other calcified tissues such as skeletal calcium, are not readily

mobilized for homeostasis during times of stress; consequently chemical analysis creates a permanent record of habitat use by an individual fish (Campana 1999). As a Pacific herring moves among PWS fjords and bays, the trace element content of the water is recorded in the otolith. Otoliths are formed in the latter part of the egg stage on the natal grounds, and this initial deposition becomes the core of the otolith (Wright et al. 2002). As the juvenile Pacific herring grows, it accretes bands of new material, which surround its original otolith core deposit. The otolith edge represents the capture location or nursery bay of sampled juvenile Pacific herring. Daily bands and yearly bands are accrued as layers much as a tree accumulates annual rings; thus, otoliths are highly suitable for age determination (Campana & Thorrold 2001; Wright et al. 2002).

Otolith band chemical composition has been utilized to identify past habitat use of fish (Thresher 1999; Campana & Thorrold 2001; Rooker et al. 2003). During crystallization, divalent cations of similar ionic radii to calcium can be substituted in the otolith matrix or trapped interstitially in the protein (Campana et al. 1995). Substitution and incorporation mechanisms of trace metals into the otolith are a function of the ionic abundances of the water they inhabit and to a lesser extent of the abiotic (i.e. ion concentration of water, temperature) and biotic factors (i.e., diet, fish growth rate) (Thresher 1999). Therefore, past habitat use of fish can be inferred by retrospectively examining the chronology of otolith chemistry from core to edge. The chemical signatures of different coastal habitats may vary over spatial scales on the order of hundreds of meters, as

demonstrated in reef fish (Dove et al. 1996; Patterson et al. 2004a,b) to scales of hundreds of kilometers (horizontal) in Icelandic cod, *Gadus morhua* (Jonsdottir et al. 2006). The organization of similar otolith chemical signatures into loose spatial groups is common, as with nursery habits of juvenile California halibut (*Paralichthys californicus*) (Forrester & Swearer 2002). Furthermore, the otolith signatures of the member regions within these groups may change due to interannual variation, as was shown in the nursery habitats of reef fish (Gillanders 2002). Otolith chemistry can vary temporally with changes in precipitation (Chesney et al. 1998), growth rate (Thresher 1999), and physical characteristics of the ambient water (Bath et al. 2000; Martin & Thorrold 2005).

Impediments to Pacific herring recovery in PWS are not well understood (Cooney et al. 2001; Norcross et al. 2001). The distribution and habitat use of early life stages and their influence on stock recovery is relatively unknown. For example, Pacific herring survival is influenced most significantly during the larval stage (Norcross et al. 2007), but little is known about this critical period. A basic understanding of the early life history of Pacific herring may aid researchers' understanding of potential stock recovery mechanisms. To date, no effective method for studying the connection between spawning and nursery locations for Pacific herring in PWS exists. Otolith chemical analysis could provide a spatial description of where individual Pacific herring were spawned.

The objective of this study was to identify broad spawning regions that contribute more recruits to nursery bays in PWS. Use of otoliths makes direct

sampling of PWS seawater unnecessary. The chemical signatures of otolith core and edge reflect the chemical signatures of natal areas and nursery bays, respectively. In this study the otolith edge signature is a proxy for the water chemistry in the nursery bay because the capture location is known and the otolith chemistry can be used as a control. By comparing otolith edge signatures with otolith core signatures, a proxy for the chemistry of the unknown natal area, the large spawning area of individual Pacific herring can be inferred. I hypothesized that the distribution of herring to nursery bays would be equivalent for each of the larger spawning regions within PWS.

## Methods

Juvenile Pacific herring were collected within PWS (Figure 1) during the Sound Ecosystem Assessment (SEA) project from 1995 to 1997 (Cooney 1999; Cooney et al. 2001; Norcross et al. 2001). For the present study, a juvenile was defined as a fish that has not joined an adult school and is isolated in a nursery bay, i.e., within its first two years of life. Frozen herring (n=626) from 11 nursery bays existed after a decade of cold storage (-18°C) in the Fisheries Oceanography Laboratory at University of Alaska Fairbanks and were used in this investigation (Table 1). These fish are not representative of the total juvenile Pacific herring collected, because a complete sampling design was not used. Each year of the three years, Eaglek Bay, Simpson Bay, Whale Bay, and Zaikof Bay were sampled (Figure 1). In 1995 otoliths were sampled from seven nursery

bays, while in 1996 and 1997, nine and four bays were sampled, respectively. Sampled Pacific herring were not from all the juvenile nursery bays in PWS described by Brown et al. (2002). The months in which samples were collected varied each year with no month consistently represented from 1995 to 1997. In 1995, samples were collected in October and November; in 1996 samples were collected in March and from May to July; and the samples from 1997 were collected in various months from February to August (Table 2). Age composition varied in each of the years and nursery bays (Table 1). Juvenile samples were represented by age-0, age-1, and age-2 with some age classes absent in some nursery bays.

Otoliths were extracted, processed, and aged prior to trace element analysis. Sagittal otoliths were extracted from intact frozen juvenile Pacific herring in a clean environment using standard techniques (Campana et al. 1995; Campana 1999; Bickford & Hannigan 2005). Thin lateral sections of the otolith were cut using a Beuhler isomet low speed saw to expose otolith core and edge portions. Otoliths were mounted onto slides with crystal-bond thermal glue and polished. Annuli were counted with an optical microscope for each otolith (Wright et al. 2002), and each individual was assigned an age and year class. Quality control of age estimates was accomplished by comparing the estimates obtained by three independent otolith readers.

Otolith composition analysis, data calibration, and normalization of the sample data were conducted on each Pacific herring sample. Trace element

analysis was conducted using a Laser Ablation (LA; New Wave UP 213nm Nd:YAG) Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Agilent 7500c). All Pacific herring otoliths were analyzed with a laser spot size of 25µm set to cycle at 10hz. A continuous line across the core and along the otolith edge was ablated, and raw elemental counts were recorded. The isotopes $^{86}Sr$, $^{87}Sr$, $^{88}Sr$, $^{24}Mg$, $^{138}Ba$, $^{44}Ca$, and $^{48}Ca$ were assessed for relative abundance per sample and calibrated to a standard of known composition (National Institute of Standards and Technology NIST 610) to limit the error associated with instrument drift over a sampling period of 1-4 hrs. GEO Pro™ v1.00 (CETAC Technologies 1999) software corrected for instrument drift by assuming a linear relationship between the standard of known consistency and the otolith chemical composition. Three replicate standards were run at the beginning of each data recording session, and a single standard was run after every ten samples. Abundances of raw chemical data were calibrated to the abundance of $^{44}Ca$ found in the standard. Isotopes were separated by weight, and the estimated counts were integrated over time for each sample. Isotope calibrated and corrected counts were normalized as ratios $^{88}Sr/^{48}Ca$, $^{24}Mg/^{48}Ca$, and $^{138}Ba/^{48}Ca$. The isotope ratio $^{87}Sr/^{86}Sr$ was used as chemical abundance information to increase the spatial resolution of otolith signatures as has been done for a different species (Barnett-Johnson et al. 2005). The isotope $^{44}Ca$ was used in the calibration of the chemical abundances to a standard of known composition, therefore, it cannot be used again in the abundance ratios or all of the ratio

values would be normalized to the standard values and not the otolith sample values. All four chemical ratios, $^{87}Sr/^{86}Sr$, $^{88}Sr/^{48}Ca$, $^{24}Mg/^{48}Ca$, and $^{138}Ba/^{48}Ca$, were used in herring otolith sample analysis (n=626).

Otoliths grow similarly to tree rings with consecutive annuli; thus by subtracting the laser width from the total diameter of the otolith, one can estimate the time represented by the ablated otolith portion. Each time a laser was shot at an otolith, an amount equal to the width of the laser was ablated or vaporized. By comparing the laser width, the age, and the distance across the sagittal otolith, an estimate was made of the amount of time that was represented by the ablated material. Measurements were made on the transverse side of the otolith thin section, across the ventral half of the posterior face with a Scion™ Color Digital CFW-1308C measuring scope from the core to the edge of the otolith. Randomly selected otoliths (n=30) were measured for ($l_o$) length across the otolith from core to edge, and lengths were divided by ($t_a$) the age. The average lengths were assumed to be proportional to the laser width (25µm) divided by ($t_i$) the time integrated by the ablation. The estimate of the amount of time represented by the ablation was:

$$\frac{\overline{l_o}}{t_a} = \frac{25\mu m}{t_i}.$$

It was estimated that the ablated material from the juvenile Pacific herring otolith edge represented the integrated regional chemical signature of habitat used for two weeks prior to capture.

Principal components analysis (PCA) (Chatfield & Collins 1980) was used to reduce the number of variables in these chemical data from the otolith edges. Statistical tests of otolith chemical abundances were conducted on the principal components, PC1 and PC2, created from the ratios $^{87}Sr/^{86}Sr$, $^{88}Sr/^{48}Ca$, $^{24}Mg/^{48}Ca$, and $^{138}Ba/^{48}Ca$. One-way ANOVA (SAS v9.1™) (Barr & Goodnight 1971) was used to make inferences about nursery bay signatures in PWS. The analysis was limited to PC1 and PC2. The principal components are uncorrelated indices (Manley 1994) of the habitat signatures created from chemical data combinations so that PC1 explains more variation than PC2. The following independent variables were examined sequentially: bay of capture, year of capture, fish year class, and capture month. Multi-factor ANOVA, of PC1 and PC2, could not be performed because there were too many missing values when including nursery bays, year class, and sampling years. The Tukey-Kramer honestly significant differences (HSD) post-hoc test ($\alpha$=0.05) was used to differentiate pairs of nursery bays in a given year, pairs of years given a particular nursery bay, and pairs of collection months within a given collection year in a particular nursery bay.

Hierarchal cluster analysis (HCA) (Everitt 1980) was used to group nursery bays with similar chemical signatures. The HCA (Clarke 1993, Clarke & Warwick 2001) used similarity coefficients calculated between all pairs of juvenile Pacific herring otolith samples from the ratios $^{87}Sr/^{86}Sr$, $^{88}Sr/^{48}Ca$, $^{24}Mg/^{48}Ca$, and $^{138}Ba/^{48}Ca$. These similarity coefficients were compared by nursery bay and

capture year (Mantel 1967; Clarke 1993; Gillanders & Kingsford 2000). The resulting dendrogram was used to assign each nursery bay to a region of similar nursery bays. HCA and PCA ordination were used to infer groups of similar nursery bay signatures. One way ANOVA ($\alpha$=0.05) and the cross validation test of a discriminant function analysis (Cover & Hart 1967) were used to confirm that the regional groups were significant. Identical analysis was conducted on the otolith cores and edges accreted in 1996 to control for interannual variation in otolith chemistry (Gillanders & Kingsford 2000).

For each nursery bay, larvae were inferred to have been either retained from the local spawning region or advected from other spawning regions, thus the contributions of spawning regions were estimated. Quadratic discriminant function analysis (QDFA) (Cover & Hart 1967) was used to discriminate among otolith edge signatures, which are proxies for the regional chemical groups of nursery bays, and to infer group membership of otolith core chemical signatures, which are proxies for natal areas. QDFA does not assume equal covariance and was applicable to these data (Manley 1994; Gillanders & Kingsford 2000). A cross-validation test was used to validate the QDFA by quantifying the correct classification of otolith edge chemical signatures from known collection bays. The natal origins of individual Pacific herring were inferred when the QDFA classified individual otolith core signatures to similar signatures of regional groups comprised of known collection bays.

The QDFA was used to classify otolith core signatures to natal origin in two ways. In this test the chemical signatures were both deposited during the same year, thus controlling for interannual variation in regional chemical signatures. The QDFA was restricted to the nine nursery bays sampled in 1996 (n=275); thus the resolution of the Pacific herring chemical signatures increased from two to three significantly different regions. For both analyses, larval retention was inferred if natal area proxies were classified as similar to the local chemical signatures. A similar chemical analysis was conducted on adult otoliths (Appendix A).

To test the ability of the QDFA to discriminate among nursery bay signatures, a cross validation sensitivity test was conducted on the nursery bay proxies sampled from 1996 otoliths. The goal of this test was to show if the chemical signatures of otoliths from Rocky and Zaikof nursery areas could be distinguished from each other. Nursery bay signatures from otolith edge chemistry collected in Eaglek and Simpson Bays were removed from the data set to test for increased spatial resolution of the regional chemical signatures in southwest PWS.

## Results

Significant differences ($p \leq 0.05$) were found in the edge chemical signatures of juvenile Pacific herring otoliths among bays, capture years, year classes and capture months (Table 3). Year-class differences among otolith edge signatures were not significantly different when the analysis was restricted

by capture year and capture bay. The chemical signatures of herring otoliths were grouped by bay and capture year and identified similar regional groups of nursery bays (Table 3).

The otolith chemical ratios, $^{87}Sr/^{86}Sr$, $^{88}Sr/^{48}Ca$, $^{24}Mg/^{48}Ca$, and $^{138}Ba/^{48}Ca$, used in the PCA, reduced the dimensionality of the data into two principal components (PC1 & PC2). The original chemical variables had substantial loading ($>|0.40|$) on at least one factor, PC1 or PC2 (Table 4). In PC1, chemical ratios $^{138}Ba/^{48}Ca$, $^{87}Sr/^{86}Sr$, and $^{88}Sr/^{48}Ca$ drove the component. Chemical ratios $^{138}Ba/^{48}Ca$ and $^{88}Sr/^{48}Ca$ were of the same sign and magnitude indicating an averaging while $^{87}Sr/^{86}Sr$ was different in sign. In PC2, $^{24}Mg/^{48}Ca$ was the factor with the greatest magnitude. These two components account for 99% of the variance, PC1 (64% of variance) and PC2 (35% of variance), of these multivariate data in two directions of orthogonal space, planes at right angles to each other.

Regional groups of similar nursery bay proxies were identified from otolith edge signatures by comparing HCA dendrograms (Figure 2) and PCA (Figure 3) ordinations. These figures show differences in the chemical signatures of otoliths from nursery bays that persisted for three years: inner (Dangerous Passage, Eaglek Bay, Galena Bay, Green Island Jack Bay, Nellie Juan, Paddy Bay, and Rocky Bay) and outer (Simpson Bay, Whale Bay, and Zaikof Bay) PWS (Figure 1). All Pacific herring natal signatures (n=626) were classified to two regional groups of nursery bays, inner and outer PWS. Second, natal signatures of otolith

cores (n=76) from fish hatched in 1996 were classified to three regional chemical groups of bays: region A, region B, and region C, identified from the 1996 otolith edge signatures. The principal component ordination shows that while the inner and outer bays vary yearly in PC2, the split is persistent through the study period for PC1. Regional chemical groups of nursery bays were identified if the otolith edge signatures within a group of nursery bays were more similar to each other than to the nursery bays within other regional groups and if there were significant differences ($\alpha$= 0.05) between or among groups (Table 3). HCA and PCA results disagree on the group assignment of Nellie Juan. Nellie Juan was assigned to the bays of inner PWS due to the higher cross validation rates in the QDFA (inner 73%).

Restricting the analysis to the abundant 1996 samples resulted in an increased spatial resolution of otolith chemical signatures (Figure 4). There were significant differences in the otolith edge signatures of Pacific herring collected in three regional groups of nursery bays (Table 3). When the analysis was restricted to 1996 (n=275), the chemical signatures separated into three significant ($p<0.001$) regional groups of nursery bays (Figure 4): region A (Eaglek Bay); region B (Dangerous Passage, Jack Bay, Nellie Juan, Paddy Bay and Rocky Bay); and region C (Simpson Bay, Whale Bay and Zaikof Bay) (Figure 1).

Following a one-way ANOVA by nursery bay, a multiple comparisons test found significant ($p\leq0.05$) differences in PC1 for the otolith edge signatures of Eaglek Bay compared to those of Simpson Bay, Whale Bay, and Zaikof Bay in

1995, 1996, and 1997. Significant differences ($p \leq 0.05$) for PC2 were indicated among the bays (Dangerous Passage, Nellie Juan, Paddy Bay, and Rocky Bay) sampled only in 1996 and the remaining PWS nursery bays. There were also significant differences ($p < 0.001$) in the edge signatures of Pacific herring otoliths collected in nursery bays sampled in multiple years (Appendix B). Despite this lack of consistency in otolith edge signatures of individual nursery bays through time, the inner and outer nursery bay pattern in otolith edge signatures was seen in all capture years regardless of the number of bays sampled (Figure 3).

A one-way ANOVA and multiple comparisons test found significant ($p \leq 0.05$) differences in the otolith edge signatures for different fish capture months within a capture year for some nursery bays (Appendix C). The PC1 was significantly different between the collection months in Simpson (1996: May and June; 1997: May and July), Whale (1997: May and August), and Zaikof (1996: February and March) Bays. Monthly variation in chemical signatures within bays was significantly different for the PC1 in most tests (Appendix C), despite the missing collection months. The PC2 was significantly different only in the July and August collection months from Eaglek Bay in 1997 (Appendix C). Most monthly variation in chemical signatures of was found in PC1 despite the consistent patterns (1995-1997) in annul regional signatures of PC2 (Figure 3).

The percentages of Pacific herring inferred to have been captured in the same region as they were hatched was different among nursery bays. Of the 301 Pacific herring captured in the inner nursery bay region, 93% of the edges

were correctly reassigned regional membership to the known capture location by the cross validation of the QDFA model. Similarly, of the 325 Pacific herring captured in the outer nursery bay region, 82% of the edges were correctly reassigned to the same known region where they were captured. The QDFA assigned the unknown chemical signatures from all Pacific herring cores (n=626) to the known regional groups established by the known edge signatures. The results indicated that 73% of Pacific herring captured in an inner nursery bay region had core chemical signatures similar to the edge signature of the inner region. Similarly, 75% of Pacific herring caught in the outer region were inferred to have natal origins in the outer region. The amount of fish inferred to have been captured in the same region as they were hatched was not equal for each nursery bay: Zaikof Bay (99%), Whale Bay (97%), Dangerous Passage (93%), Galena Bay (90%), Paddy Bay (88%), Rocky Bay (88%), Jack Bay (84%), Eaglek Bay (80%), Nellie Juan (77%) and Green Island (44%) and Simpson Bay (25%).

Analysis of the otolith core (n=76) and edge (n=275) material accreted in 1996 was necessitated by the significant interannual variation in otolith edge chemistry (Appendix B). Three significantly different chemically similar regions (Table 3) were identified in the 1996 sample data (Figure 4). The Pacific herring samples from the nine bays sampled in 1996 were split into three significantly different regional chemical groups resulting in lower rates of discrimination among regions (Table 5) but higher spatial resolution of chemical signatures than inner and outer PWS. The otolith data show that larvae from region B

(Dangerous Passage, Jack Bay, Nellie Juan, Paddy Bay and Rocky Bay), contribute the most juveniles to both Eaglek Bay (70%) and Simpson Bay (63%). Furthermore, juvenile Pacific herring originating in one part of region C (Whale Bay, Zaikof Bay) tended to stay in the region C nursery bays (Table 6). Natal otolith signatures similar to region A (Eaglek Bay) were not identified in the core signatures of Pacific herring caught in Whale or Zaikof Bays (Table 6). Otolith edges that were similar to otolith cores were estimated by chemical region: A (14%), B (N/A), C (48%). No 1996 year class Pacific herring exist from region B, but when the core chemical signatures of all sample years were pooled and classified to the three chemical regions, 70% of region B juveniles had similar signatures to the natal areas of region B (Appendix D).

Sensitivity analysis results showed no increase in spatial resolution of the QDFA when the 1996 Pacific herring samples were limited to the southeast portion of PWS. The QDFA discriminated between the edge signatures of Pacific herring in Rocky and Zaikof Bays. The chemical signatures of Rocky Bay were most similar to region B bays with only 6% misclassification to Zaikof Bay (Table 7). Similarly, the chemical signatures of Zaikof Bay were distinct from region B bays, resulting in no misclassifications in any of the nursery bay proxies for region B (Table 7). The chemical signatures of Rocky Bay otolith edges were equivalent to all nursery bay proxies in region B (Table 7).

## Discussion

This study demonstrates that larvae are not distributed randomly from spawning grounds to nursery bays. Regional groups of nursery bays with similar otolith chemistries enabled inference of natal location and approximation of larval contributions of broad spawning regions to nursery bays. Nursery bays accumulate larvae that are advected from distant natal regions or they 'retain' larvae within broad areas of PWS. The larval 'retention' described in this study refers to broad areas of PWS that share chemical similarities in otolith chemistry and does not refer to larval retention in distinct geographically and temporally stable retention areas (Iles & Sinclair 1982), which has led to the reproductive isolation and speciation of Atlantic herring (*Clupea harengus*) (Sinclair & Iles 1985).

This is the first study that utilizes otolith chemistry to retrospectively infer the natal location of juvenile Pacific herring in PWS, which improves understanding of the source of larvae to nursery bays from our present knowledge. The chemical signatures of otolith edges were used as controls because the capture location of the herring was known. Furthermore, juveniles have been isolated in their nursery bays since metamorphosis (Stokesbury et al. 2000) and the otolith edge chemistry reflects trace elements in the ambient seawater. Juvenile Pacific herring from different regional nursery bay groups within PWS have differences in their otolith edge chemical signatures due to the chemical composition of the water source.

Previous locations of Pacific herring in PWS were inferred from the known chemical signatures of otolith edges. As in similar studies using regional otolith signatures to understand the sources of recruits (Swearer et al. 1999; Gillanders & Kingsford 2000; Forrester & Swearer 2002; Gillanders 2002), this study infers the natal origin of individual fish by examining isotope signatures of otolith cores. If an otolith has similar natal (core) and nursery (edge) chemistry, it implies that the fish was spawned and collected in the same region or that the water in the two regions was similar (Stransky et al. 2005). Similarity in core and edge chemistry implies that for PWS, larval retention exists in broad regions of nursery bays. If the natal area chemistry and nursery bay chemistry are not similar, it indicates that larvae originated in another chemically dissimilar region or that the water chemistry has changed overtime. In this study, not all possible natal and nursery areas were sampled in PWS; therefore, it cannot be concluded that other chemically similar regions did not contribute larvae to nursery bays. However, if negligible advection of larvae into PWS from without is assumed, it is possible to estimate a natal area's contribution to the juvenile members of different nursery bays in terms of the amount of retained larvae to nursery bays from the local spawning regions.

The inner and outer regions indicated by otolith signatures resemble the chemistry of the source water composition and are not significantly influenced by temperature and salinity characteristics. Otolith trace element composition is believed to be influenced by the temperature and ionic abundance of all elements

of the source water (Fowler et al. 1995; Thresher 1999), but these otolith data do not support that conclusion. Physical oceanographic data for the four bays sampled in all years do not explain the otolith chemistry. These bays vary in physical characteristics such as: basin area, drainage area, depth, ionic abundance, and temperature (Gay & Vaughan 2001). The two deepest bays (Whale and Zaikof) had similar physical and chemical characteristics during this time, while Simpson Bay had characteristics similar to Eaglek Bay (Gay & Vaughan 2001). In contrast, the otolith data indicate distinct inner and outer nursery bay chemistries in PWS. Disparities indicate that the chemical signatures of regions are being influenced by different source water ionic abundances or by biotic factors such as diet and growth rate or a combination of factors (Thresher 1999). GOA transport of water (Niebauer et al. 1994; Gay & Vaughan 2001; Vaughan et al. 2001) and zooplankton (Kline 1999) has been noted in the outer bays such as Simpson, Whale, and Zaikof, while Eaglek Bay is the most isolated from GOA (Gay & Vaughan 2001). Proximity to the GOA is most likely affecting otolith chemical composition in the inner and outer nursery bays of PWS. Similarly, otolith chemical signatures of protected bays and open-coast nursery habitats were successfully discriminated for California halibut (Forrester & Swearer 2002).

In PWS, precipitation and surface water temperature vary seasonally and annually, which could cause temporal variation in otolith signatures. Temporal variation in otolith chemistry is likely due to these seasonal salinity and

temperature changes that can distort spatial resolution of otolith chemical analysis by altering the ionic composition of water in the nursery bays and by altering fish growth rates (Bath et al. 2000; Gillanders 2002; Martin & Thorrold 2005). From 1995 to 1997, the period from which otoliths were analyzed, PWS received between 3.5 to 5.4 m of rain annually with maximum precipitation from April to September and minimum precipitation from October to March (Gay & Vaughan 2001). Annual variation in surface water temperatures varied for each nursery bay over the sampling period with Eaglek Bay having the highest seasonal variability (13.5° C annually). The otolith signatures used in this study represent the variation caused by seasonal temperature and salinity fluctuations in PWS from February through December. Future sampling efforts should control for seasonal variation by focusing on Pacific herring from a particular month or season to facilitate spatial resolution of chemical proxies.

The otolith chemical signatures of PWS nursery bays are temporally variable, and comparing core and edge signatures created during the same year and season is advantageous. Attempts to investigate temporal variability at the month scale were confounded by the missing collection months; therefore, one cannot make inferences about temporal variation on a scale under a year. Monthly differences in otolith edge signatures vary predominantly in PC1 (Appendix C), while the significant interannual variation (Table 3) is found in PC2 (Figure 3), indicated by circles. Nevertheless, it is likely that interannual variation in otolith chemistry is influenced by monthly variation. Causes of

interannual variation are unclear but fluctuations in Sr and Ba otolith chemistry have been linked to changes in terrestrial runoff (Thorrold & Shuttleworth 2000). Therefore, interannual variation in otolith chemical signatures could be caused by changes in glacier and river runoffs, which are highly dependent on temperature, insolation, and precipitation. Concentrations of Mg in otoliths can vary both with growth and otolith accretion rate changes (Martin & Thorrold 2005); thus, annual changes in PWS's ecology could cause interannual variability in otolith chemistry.

The spawning origin of juvenile Pacific herring was inferred despite the lack of a pre-planned sample design for otolith collection. Pacific herring samples do not represent all locations in PWS but were collected from important nursery bays. The lack of known edge chemical signatures from all areas could lead to the erroneous classification of the unknown core signatures in the QDFA. In this study, spatial resolution of chemical signatures is unclear because, although nursery bays were pooled in two groups with similar signatures, the groups do not have definitive physical boundaries. It is probable that otolith signatures vary on scales smaller than the inner and outer collection of similar nursery bays and that intense sampling effort could reveal individual nursery bay signatures. Inadequate sampling can lead to inappropriate interpretations of otolith signatures (Dove et al. 1996; Gillanders & Kingsford 2003; Patterson et al. 2004a). Future studies should clarify the scale of regional chemical signatures; such studies should focus collection efforts on areas utilized by Pacific herring during both their natal and juvenile stages.

A year-class record of nursery bay proxies would enable researchers to understand the previous habitat use of juvenile and adult Pacific herring originating in PWS; therefore, Pacific herring samples must be collected annually. Such year-class 'libraries' of elemental fingerprints in otoliths have been suggested by Gillanders & Kingsford (2000) to track movements of adult reef fish from natal to nursery areas. By analyzing the chemical composition of the otolith core, a retrospective of Pacific herring spawning areas can be created. By comparing trace element composition of the core otolith layer to the appropriate year-class library, inferences can be made about the spawning grounds of both juvenile and adult fish. Attempts to infer spawning ground signatures from spawning adults were inconclusive (Appendix A); therefore, ,juvenile Pacific herring collections should be made annually during the spawning season, creating a library of otolith chemistry that could enable researchers to examine the scope of temporal changes in chemistry.

A year-class library was utilized to infer the natal location of Pacific herring from the otolith material accreted in 1996. Annual variations in otolith chemistry of nursery bays were removed from this analysis because otolith edges of juveniles captured in 1996 (n=275) were accreted in the same year as otolith cores accreted in Pacific herring that hatched in 1996 (n=76). This analysis yielded different conclusions about larval sources for nursery bays in PWS than by pooling otolith data from 1995 to 1997. The chemical signature of few otolith cores matched edge signatures of the four intensively studied bays (Eaglek Bay,

Simpson Bay, Whale Bay, and Zaikof Bay); therefore, it appears that other areas are the main larval source to PWS Pacific herring. The importance of region B as a source of larvae to Eaglek and Simpson Bays can be inferred from otolith data. From 1995 to 1997 the Alaska Department of Fish and Game Pacific herring spawning surveys (Morstad et al. 1998) did not document any spawning areas in region B other than Rocky Bay, suggesting that region B had other undocumented spawning areas, as were historically noted in Dangerous Passage, Nellie Juan, and Paddy Bay (Brown et al. 2002). Alternatively, it might indicate that spawning grounds in Rocky Bay contribute to juvenile PWS Pacific herring.

Variability in otolith edge chemistry can be influenced by the period of time that is represented by the otolith material used during laser ablation. During the trace element analysis of otoliths, a 25μm wide line was ablated that integrates the chemical composition of habitats utilized by Pacific herring over the previous two weeks. Juvenile Pacific herring are thought to remain isolated in nursery bays for two years (Stokesbury et al. 2002), however, the nursery bay proxies of Pacific herring captured in Rocky Bay were equally similar to all nursery bays in region B. This may indicate movement between bays within region B in the two weeks prior to capture. However, fish occupying different habitats with similar chemical signatures (Stransky et al. 2005) could lead to erroneous assumptions about the spawning grounds used by juvenile Pacific herring. Another possible inference is that Pacific herring occupy habitats within Rocky Bay that resemble

the chemically similar region B nursery bays. A study of juvenile staghorn sculpin (*Leptocottus armatus*) habitat use in Pacific Northwest estuaries indicated that spatial environmental variability within nursery habitats can result in the classification of an otolith signature to the wrong nursery area (Miller 2007). Future juvenile Pacific herring sampling efforts in PWS should examine the spatial variability within nursery bays by sampling Pacific herring occupying the various arms and drainages of each nursery bay.

Habitat of Pacific herring can be retrospectively understood through the use of otolith chemical signatures corresponding to the natal and nursery areas. The high precipitation rates and runoff of the coastal North Pacific support distinct otolith chemical signatures. Coastal spawning and nursery areas of other commercially and ecologically important species can be retrospectively inferred by these habitat proxies. Identification and protection of spawning areas and nursery habitats that contribute to the successful recruitment of fish could help to rebuild depressed stocks.

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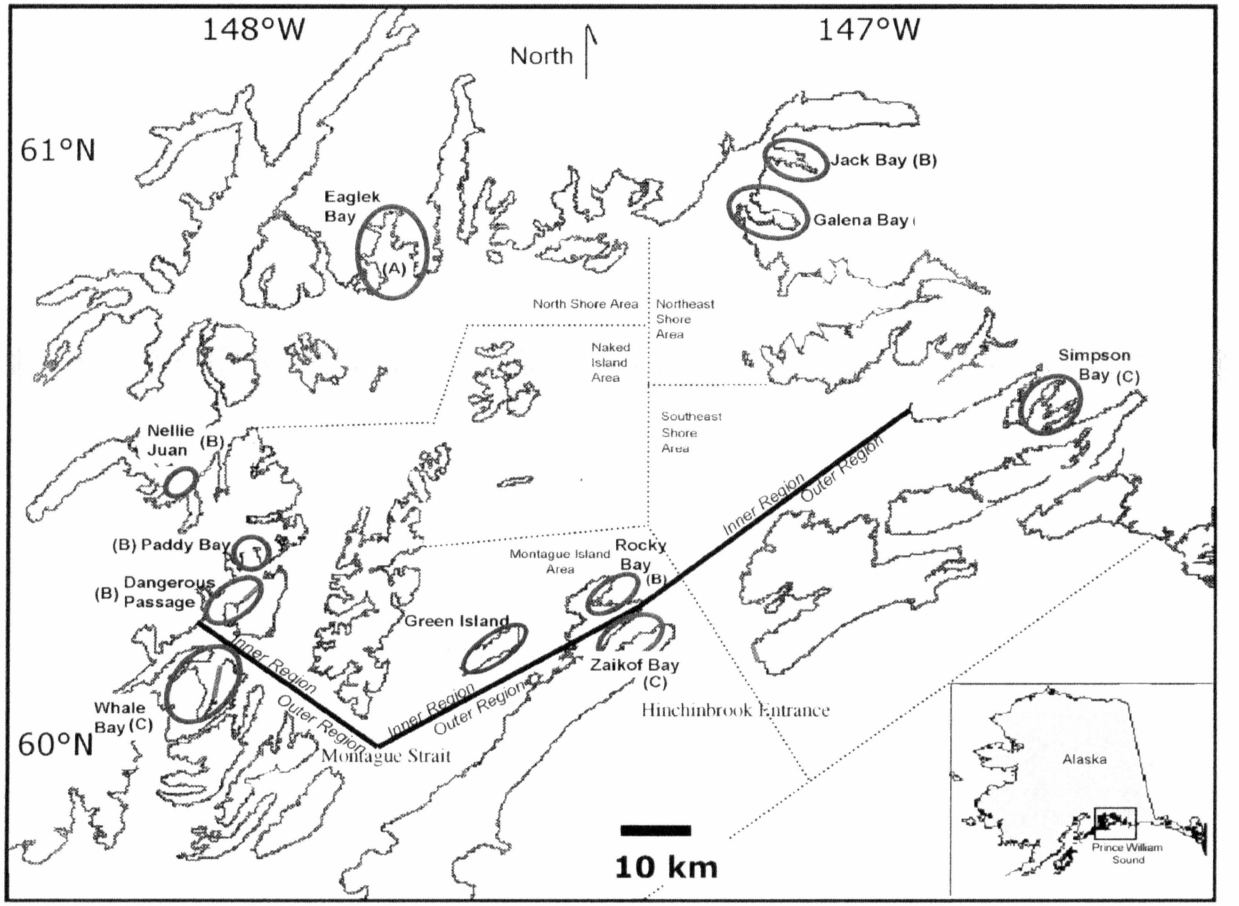


Figure 1. Map of Prince William Sound, Alaska, showing Pacific herring capture bays (circled areas) and management areas (dotted lines). Also shown are the chemical regions identified from the otolith edge signatures: inner, outer; in parentheses the 1996 regions A, B, and C.

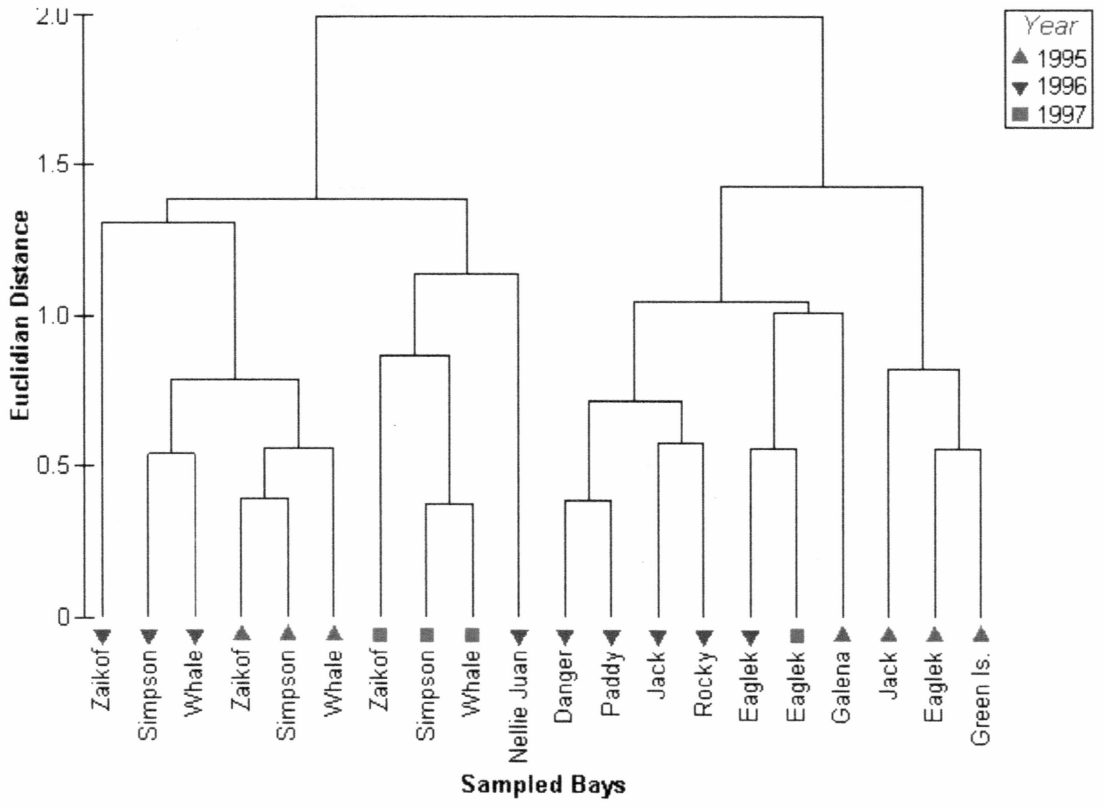


Figure 2. Hierarchal cluster analysis (HCA) dendrogram of juvenile Pacific herring nursery ground otolith signatures restricted by capture year. The dendrogram shows the similarities of the regional chemical patterns of Pacific herring nursery bays through time. Nellie Juan appears to group with the bays of outer PWS in the HCA only.

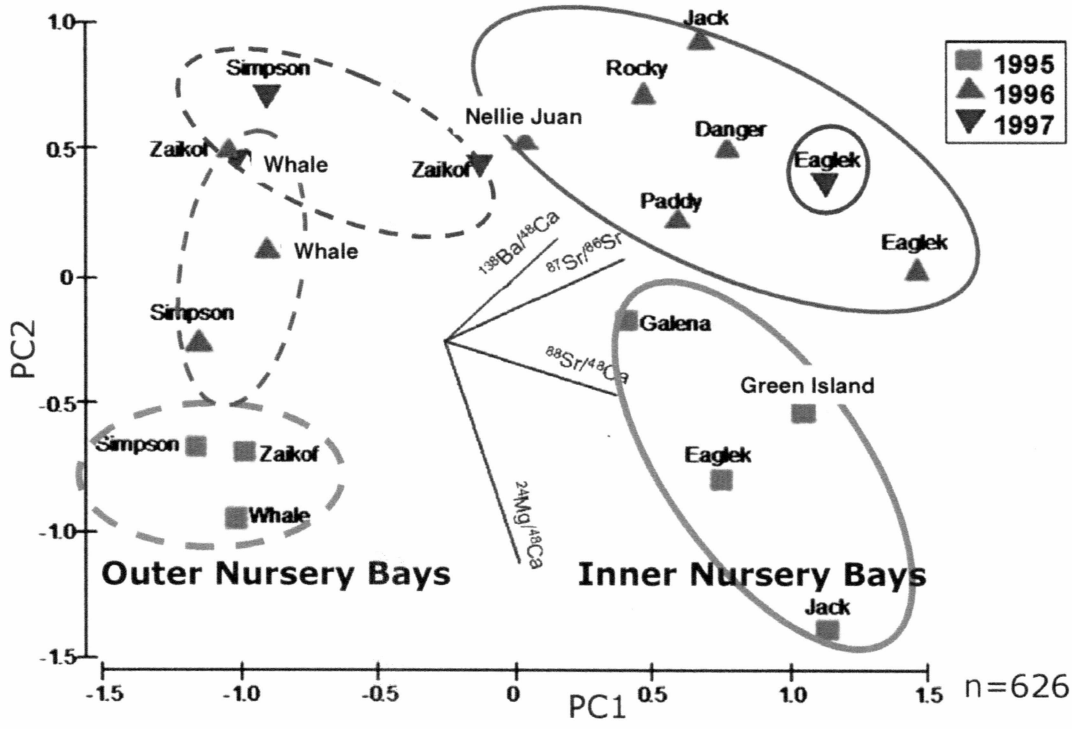


Figure 3. Principal component analysis (PCA) ordination of 1995-1997 juvenile Pacific herring nursery ground otolith signatures used to compare the similarities of the otoliths signatures from nursery bays in which the Pacific herring were captured by the years Pacific herring were collected. Overlaid are the vectors of influence that each chemical ratio, $^{88}Sr/^{48}Ca$, $^{24}Mg/^{48}Ca$, $^{138}Ba/^{48}Ca$, $^{87}Sr/^{86}Sr$ has on the ordination. Circles represent the regional groups of similar chemical signatures; dashed lines indicate outer region, solid line indicates inner region.

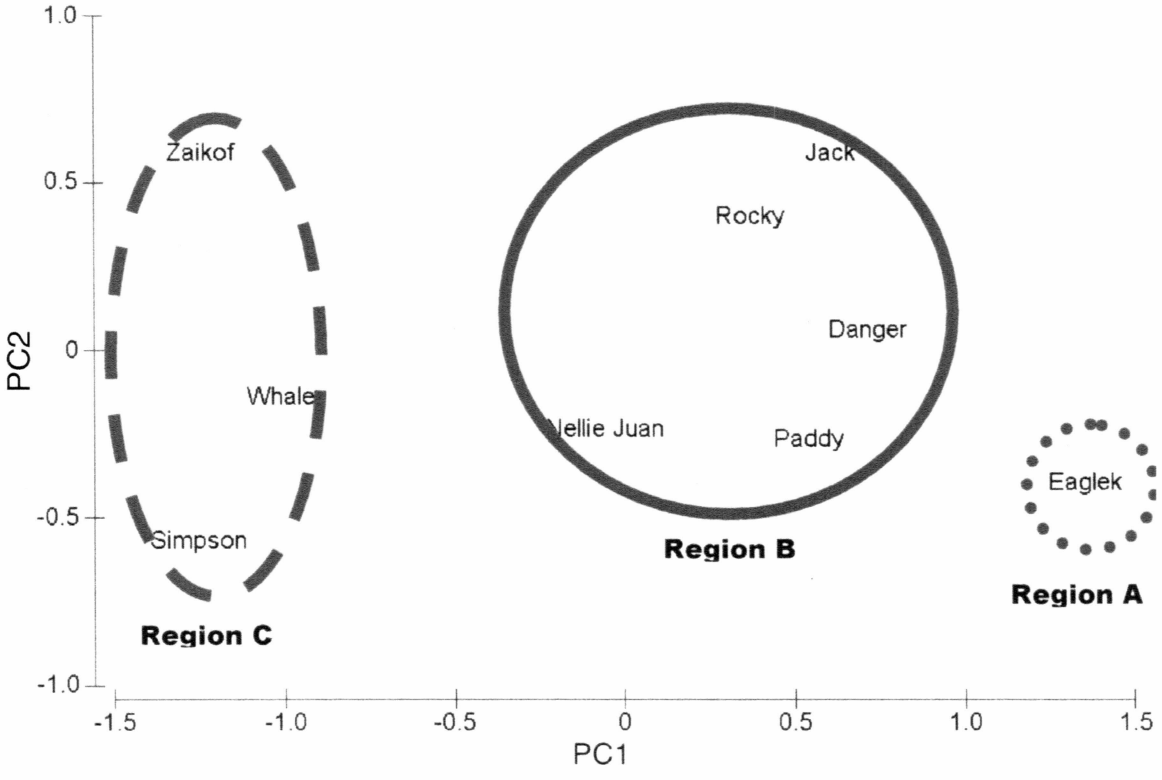


Figure 4. Principal component analysis (PCA) ordination of 1996 juvenile Pacific herring nursery ground otolith signatures used to compare the similarities of the otoliths signatures from nursery bays in which the Pacific herring were captured. Circles represent the regional groups of similar chemical signatures; dashed lines indicate region C, solid line indicates region B, doted line indicates region A.

Table 1. Capture location in Prince William Sound, Alaska and number of Pacific herring used for otolith chemical analysis. Otolith samples are listed by management area, capture bay, capture year, and the fish year class.

| Bay | 1995 | | | | 1996 | | | | 1997 | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age-0 | Age-1 | Age-2 | Total | Age-0 | Age-1 | Age-2 | Total | Age-0 | Age-1 | Age-2 | Total | **163** |
| Green Is. | 28 | 4 | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 |
| Rocky | 0 | 0 | 0 | 0 | 0 | 7 | 10 | 17 | 0 | 0 | 0 | 0 | 17 |
| Zaikof | 24 | 6 | 3 | 33 | 0 | 12 | 26 | 38 | 9 | 32 | 2 | 43 | 114 |
| | | | | | | | | | | | | | **156** |
| Danger | 0 | 0 | 0 | 0 | 0 | 19 | 10 | 29 | 0 | 0 | 0 | 0 | 29 |
| Paddy | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 17 | 0 | 0 | 0 | 0 | 17 |
| Whale | 19 | 0 | 0 | 19 | 0 | 44 | 0 | 44 | 24 | 9 | 14 | 47 | 110 |
| | | | | | | | | | | | | | **147** |
| Eaglek | 27 | 11 | 0 | 38 | 0 | 38 | 2 | 40 | 2 | 36 | 5 | 43 | 121 |
| Nellie | 0 | 0 | 0 | 0 | 0 | 3 | 23 | 26 | 0 | 0 | 0 | 0 | 26 |
| | | | | | | | | | | | | | **59** |
| Galena | 0 | 20 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
| Jack | 14 | 5 | 0 | 19 | 0 | 19 | 1 | 20 | 0 | 0 | 0 | 0 | 39 |
| | | | | | | | | | | | | | **101** |
| Simpson | 9 | 8 | 0 | 17 | 5 | 39 | 0 | 44 | 17 | 22 | 1 | 40 | 101 |
| | 121 | 54 | 3 | 178 | 5 | 198 | 72 | 275 | 52 | 99 | 22 | 173 | **626** |

Table 2. Number of Pacific herring samples collected in Prince William Sound by year of collection and month of collection: month (n).

| Bay | 1995 | 1996 | 1997 | Total |
|---|---|---|---|---|
| Danger | 0 | 29 | 0 | 29 |
| | | 3(18); 6(11) | | |
| Eaglek | 38 | 40 | 43 | 121 |
| | 10(18); 11(20) | 6(21); 7(19) | 7(29); 8(14) | |
| Galena | 20 | 0 | 0 | 20 |
| | 11(20) | | | |
| Green Is. | 32 | 0 | 0 | 32 |
| | 10(32) | | | |
| Jack | 19 | 20 | 0 | 39 |
| | 11(19) | 3(20) | | |
| Nellie | 0 | 26 | 0 | 26 |
| | | 3(26) | | |
| Paddy | 0 | 17 | 0 | 17 |
| | | 3(17) | | |
| Rocky | 0 | 17 | 0 | 17 |
| | | 3(17) | | |
| Simpson | 17 | 44 | 40 | 101 |
| | 10(17) | 3(20); 5(16); 6(4); 12(4) | 5(15); 7(25) | |
| Whale | 19 | 44 | 47 | 110 |
| | 10(19) | 3(18); 5(7); 6(19) | 5(23); 8(24) | |
| Zaikof | 33 | 38 | 43 | 114 |
| | 10(33) | 5(18); 6(20) | 2(17); 3(26) | |
| Total | 178 | 275 | 173 | **626** |

Table 3. One-way ANOVA comparing otolith edge data from juvenile Pacific herring by nursery bay, collection year, fish year class, fish collection month, and regions of chemically similar nursery bay otolith signatures: inner or outer PWS and 1996 Regions. Principal components one and two are represented by PC1 and PC2. Results are indicated by the following abbreviations: df= degrees of freedom; mse = mean squared error; p = p-value.

| | Nursery Bays | | | Collection Year | | | Year class | |
|---|---|---|---|---|---|---|---|---|
| | df | mse | p | df | mse | p | df | mse |
| **PC1** | 10 | 12.22 | <0.001 | 2 | 13.61 | <0.001 | 4 | 1.56 |
| **PC2** | 10 | 1.98 | <0.001 | 2 | 32.23 | <0.001 | 4 | 1.69 |

| | Month | | | Inner and Outer PWS | | | 1996 Regions | |
|---|---|---|---|---|---|---|---|---|
| | df | mse | p | df | mse | p | df | mse |
| **PC1** | 8 | 7.346 | <0.001 | 1 | 94.7 | <0.001 | 2 | 15.98 |
| **PC2** | 8 | 8.293 | <0.001 | 1 | 1.43 | 0.089 | 2 | 4.285 |

Table 4. Factor loadings from principle components analysis (PCA). Principal components one and two are represented by PC1 and PC2 for the otolith edge chemical signatures from Pacific herring samples (n=626).

| | PC1 | PC2 |
|---|---|---|
| $^{87}Sr/^{86}Sr$ | -0.660 | -0.319 |
| $^{88}Sr/^{48}Ca$ | 0.653 | -0.351 |
| $^{24}Mg/^{48}Ca$ | 0.065 | 0.857 |
| $^{138}Ba/^{48}Ca$ | 0.715 | 0.203 |

Table 5. Cross-validation test of otolith nursery ground chemical signatures from juvenile Pacific herring using quadratic discriminant function analysis.
Table shows the percentage of nursery ground otolith signatures from known geographic origin that were correctly classified by QDFA to the Pacific herring's region of capture: Region A= Eaglek Bay; Region B= Dangerous Passage, Nellie Juan, Paddy Bay, Rocky Bay; Region C= Simpson Bay, Whale Bay, Zaikof Bay; restricted to the 1996 year Pacific herring nursery signatures.

| | Region A | Region B | Region C | n |
|---|---|---|---|---|
| Region A | 63% | 30% | 7% | 60 |
| Region B | 27% | 66% | 7% | 89 |
| Region C | 8% | 10% | 82% | 126 |
| | | | Total= | 275 |

Table 6. Classification of juvenile Pacific herring otolith core chemical signatures to the regional chemical signatures of otolith edges in PWS using quadratic discriminant analysis. QDFA was restricted to 1996 cores classified to 1996 edge signatures: Region A= Eaglek Bay; Region B= Dangerous Passage, Nellie Juan, Paddy Bay, Rocky Bay; Region C= Simpson Bay, Whale Bay, Zaikof Bay;

| Region | Bay | Region A | Region B | Region C | n |
|---|---|---|---|---|---|
| A | Eaglek | 14% | 70% | 17% | 36 |
| | Simpson | 27% | 63% | 9% | 22 |
| C | Whale | 0% | 0% | 100% | 9 |
| | Zaikof | 0% | 11% | 89% | 9 |
| | | | | Total= | 76 |

Table 7. Sensitivity test for the discrimination of nursery area chemical signatures using quadratic discriminant analysis. Results of the cross validation of the QDFA for a limited set of bays sampled in 1996, the nursery area signatures of Eaglek Bay and Simpson Bay were removed from the analysis. The table shows the strong dissimilarities in the chemical characteristics of Rocky Bay and Zaikof Bay despite a close proximity.

| | Dangerous | Jack | Nellie Juan | Paddy | Rocky | Whale | Zaikof | Samples |
|---|---|---|---|---|---|---|---|---|
| Inner Bays | | | | | | | | |
| **Dangerous** | 24% | 28% | 7% | 38% | 3% | 0% | 0% | 29 |
| **Jack Bay** | 10% | 45% | 10% | 20% | 15% | 0% | 0% | 20 |
| **Nellie Juan** | 8% | 15% | 27% | 27% | 4% | 15% | 4% | 26 |
| **Paddy Bay** | 23% | 18% | 6% | 47% | 6% | 0% | 0% | 17 |
| **Rocky Bay** | 12% | 12% | 18% | 29% | 12% | 12% | 6% | 17 |
| Outer Bays | | | | | | | | |
| **Whale Bay** | 0% | 0% | 23% | 0% | 9% | 50% | 18% | 44 |
| **Zaikof Bay** | 0% | 0% | 5% | 0% | 0% | 16% | 79% | 38 |
| | | | | | | | Total= | 191 |

## Appendix A
## Otolith chemical analysis of adult Pacific herring

The life cycle of Pacific herring (*Clupea pallasii*) and the physical and chemical diversity of PWS are conducive to otolith chemical analysis. Adult pacific herring are demersal substrate spawners, which move into subtidal waters to fertilize and deposit their eggs (Hay 1990). Adult Pacific herring age-4 and older migrate in late March to spawn on 23 – 168 kilometers of coastline in PWS (Norcross et al. 2001). Adults spawn in mid-April and much of the Pacific herring eggs are lost to predation, wave-action, and exposure (Rooper et al. 1999). If Pacific herring larvae are advected from natal areas, the larvae drift through PWS pushed by surface currents, density changes, and meteorological forces (Brown et al. 1996). Metamorphosis of the larval Pacific herring begins to occur in June of that same year the Pacific herring become nektonic and swim to favorable habitats (Stokesbury et al. 2002). In August, the young Pacific herring begin to form schools and aggregate at the heads of bays far from coastal waters (Brown et al. 2002; Stokesbury et al. 2000). These populations stay isolated in their respective nursery bays until June of their second year (Stokesbury et al. 2000). At that time this cohort of Pacific herring leaves the bays and joins adult schools (Stokesbury et al. 2000).

Throughout the life of a Pacific herring the trace element content of the water is recorded in the otolith. Otolith bands are accrued during

Appendix A continued.

the fish's time of residence in the natal areas, nursery bays, and adult spawning locations, thus recording the unique spatial chemical signatures. Otoliths are single cellular crystalline deposits of $CaCO_3$, in the form of aragonite, within an otolin-1 protein matrix. There are three calcified otoliths structures found in teleosts; the sagittae is the largest and most studied (Wright et al. 2002). Otolith tissue is not reabsorbed by the body, as other calcified tissues are; it is this quality that makes otoliths unique in fish (Campana 1999). Otoliths, unlike other calcified tissues such as skeletal calcium, are not readily mobilized for homeostasis during times of stress; consequently, otoliths are highly suitable for age determination (Wright 2002) and chemical analysis (Campana 1999). Otoliths are formed in the latter part of the egg stage. The initial deposition of material becomes the core of the otolith (Wright et al. 2002). As the juvenile Pacific herring grows it accretes bands of new material, which surrounds its original core deposit. Daily bands and yearly bands are accrued as layers. The edge portion of the otolith represents the capture location of the fish. Growth is recorded as assorted bandwidths inside the otolith, much as a tree accumulates annual rings. The daily and annual bands (annuli) have long been used as detectors of age and growth rate in fish (Campana & Thorrold 2001; Wright et al. 2002).

The chemical compositions of individual bands have been used to identify past habitat use of the fish (Rooker et al. 2003; Campana & Thorrold 2001;

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Thresher 1999). During crystallization, divalent cations of similar ionic radii to calcium can be substituted in the otolith matrix or trapped interstitially in the protein (Campana et al. 1995). The mechanism of substitution and incorporation of trace metals into the otolith are a function of abiotic (i.e., temperature, salinity) and biotic (i.e., diet, fish growth rate) conditions (Thresher 1999). Therefore, movement of fish can be determined by retrospectively examining the chronology of otolith chemistry. The regional signatures may vary, as demonstrated in reef fish where otolith chemistry varied over spatial scales on the order of hundreds of meters (Dove et al. 1996; Patterson et al. 2004a,b). Spawning site fidelity would be inferred if the adult spawning ground signature were similar to the natal ground signature.

The objective of this study was to identify spawning grounds that contribute more recruits to spawning adults Pacific herring in PWS. Use of otoliths makes direct sampling of PWS seawater unnecessary. The chemical signatures of otolith core and edge reflect the chemical signatures of natal areas and spawning grounds, respectively. In this study the otolith edge signature is a proxy for the water chemistry in the spawning ground, because the capture location is known and the otolith chemistry can be used as a control. By comparing otolith edge signatures with otolith core signatures, a proxy for the chemistry of the unknown natal area, the spawning area of individual Pacific

Appendix A continued.

herring can be inferred. I hypothesized that the Pacific herring return to natal areas as adults to spawn.

Methods

For the purposes of this study an adult is defined as by age-3, generally regarded as first reproduction (Williams & Quinn 2000). Adults were captured in the spawning grounds of Two Moon and Sawmill bays. The Pacific herring samples were frozen whole and shipped to the University of Alaska Fairbanks (UAF) Fisheries Oceanography Lab.

Otoliths were extracted, processed and aged prior to trace element analysis. Sagittal otoliths were extracted from intact frozen adult Pacific herring in a clean environment using standard techniques (Bickford & Hannigan 2005; Campana 1999; Campana et al. 1995). All tools used for extraction were made of Teflon™ and were acid washed prior to use to minimize contamination. Thin sections were cut laterally across the otolith using a Beuhler isomet low speed saw to expose the core and edge portions of the otolith. The otoliths were mounted onto slides with crystal-bond thermal glue and polished. Each otolith's annuli were counted with an optical microscope and each sample was assigned an age. A year class was assigned to each sample based on the age of fish and the year the fish was captured. Quality control of age estimates was accomplished by comparing the estimates obtained by three independent

Appendix A continued.

observers. Age estimates were accepted only after 95% agreement was reached among all otolith readers.

The analysis of the otolith composition and the data calibration were conducted prior to the normalization of the sample data. Trace element analysis was conducted on the Laser Ablation (LA; New Wave UP 213nm Nd:YAG) Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Agilent 7500c) at the Advanced Instrumentation Facility on the UAF campus. All Pacific herring otoliths were analyzed using standard laser settings: laser spot size 25µm; 10hz. For the adult samples, lines were ablated and chemical abundances were measured at the core sector (natal region), middle sector (nursery ground region), and edge sector (spawning ground region) of each otolith. By comparing the laser width, the age of the Pacific herring, and the distance across the otolith, an estimate was made for the amount of time that was represented by the ablated material. The isotopes $^{86}Sr$, $^{87}Sr$, $^{88}Sr$, $^{24}Mg$, $^{138}Ba$, $^{44}Ca$, and $^{48}Ca$ were assessed for relative abundance per sample and calibrated to a standard of known composition (National Institute of Standards and Technology NIST 610) to limit the error associated with instrument drift over a sampling period of 1-4 hrs. GEO Pro™ v1.00 (CETAC Technologies 1999) software corrected for instrument drift by assuming a linear relationship between the standard of known consistency and the chemical

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composition of the otoliths.

A set of three replicate standards was run at the beginning of each data recording session, and a single standard was run after every ten samples. The processing of raw chemical data consisted of a calibration to the abundance of $^{44}Ca$ found in the standard. Peak chemical counts were integrated through time. The calibrated and corrected counts for the isotopes were normalized as ratios $^{88}Sr/^{48}Ca$, $^{24}Mg/^{48}Ca$, and $^{138}Ba/^{48}Ca$. The isotope ratio $^{87}Sr/^{86}Sr$ was used as additional chemical abundance information (Barnett-Johnston et al. 2005). All four chemical ratios were used in the analysis.

All four chemical ratios were examined collectively to infer the regional chemical signature of the capture location from the otolith's edge composition. Regional groupings of bays were determined. Adult Pacific herring habitat use was inferred from the regional signatures of the known capture location of the samples. The chemistry of adult otolith samples was compared to the juvenile regional signatures to identify possible past habitat use. The adult spawning ground signatures and natal signatures were also compared to identify spawning region fidelity, or the tendency of PWS Pacific herring to return to natal areas to spawn.

The software programs PRIMER v6 (Plymouth Routines in Marine Environmental Research) (Clarke 1993; Clarke & Warwick 2001) and SAS v9.1™

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(Barr & Goodnight 1971) were used to analyze the chemical abundance data to make inferences about the habitat use of both juvenile and adult Pacific herring in PWS. Univariate and multivariate statistical techniques were used to test for the sources of variance in the data. Factors were compared using one-way ANOVA ($\alpha$=0.05), because there were too many missing samples to do two-way ANOVA. The juvenile Pacific herring samples were explored to identify the significant differences among the independent variables: management area, regional chemical groups, bay in which juvenile Pacific herring were captured, the year in which Pacific herring were captured, and year class of the fish. Tukey-Kramer honestly significant differences (HSD) post hoc test was used to identify the significantly different instances within the bays in which the Pacific herring were captured in regards to the sources of variance and among bays with replicate Pacific herring sampling events over multiple years. One-way ANOVA tests were also conducted on the adult Pacific herring samples. The regional chemical signatures were compared among year classes and otolith sectors. The three sectors were compared between bays resulting in significant differences among the core, middle, and edge sectors of Two Moons Bay (TMB) and Snug Corner Cove (SCC).

A stepwise discriminant analysis was used to identify the best set of

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chemical data to utilize in a principal components analysis (PCA) (Chatfield & Collins 1980). All four chemical ratios were used in the PCA, which reduced the dimensionality of the data into two principal components (PC1 & PC2). These two components maximize the variance of these multivariate data in two directions of orthogonal space. The principal components are uncorrelated indices of the regional signatures created from combinations of the chemical data in such a way that PC1 explains more variation than PC2 (Manley 1994). The analysis was limited to PC1 and PC2 because they explained the majority of the variance in the data set.

PCA ordinations and hierarchal cluster analysis (HCA) (Everitt 1980) were used to identify bays with similar chemical signatures and to assign each bay to a regional chemical region within PWS. The similarity coefficients calculated between all pairs of juvenile Pacific herring otolith samples were compared by the bay and year they were captured (Mantel 1967; Clarke 1993; Gillanders & Kingsford 2000). Regional chemical groups of bays were identified if the bays within a region were more similar to each other than the bays within other regional groups. The regional groups have similar chemical signatures that relate to the relative differences in the trace element composition within and between regions in which Pacific herring were captured. A HCA dendrogram and a PCA ordination were used to infer the similarities of replicate Pacific herring

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within regional groups of bays. A regional group of bays was determined when PCA ordinations, HCA, and the cross-validation of Quadratic discriminant function analysis (QDFA) agreed.

Quadratic Discriminant Function Analysis was used to infer a regional classification of adult natal area signatures and adult nursery areas signatures to the regional chemical signatures determined from the juvenile Pacific herring captured in PWS nursery areas. The adult natal nursery signatures were classified to the inner and outer PWS regions as well as A, B, and C regional groups determined from the juvenile analysis. The habitat use of adult Pacific herring during their natal and nursery region residence time was inferred.

Results

The regional chemical signatures of adult Pacific herring were split into two significant regional groups of bays, inner and outer PWS ($p<0.001$) for 1995, 1996 and 1997 and the three significant chemical regions ($p<0.05$) inferred within PWS: Region A (Eaglek Bay); Region B (Dangerous Passage, Jack Bay, Nellie Juan, Paddy Bay and Rocky Bay); Region C (Simpson Bay, Whale Bay and Zaikof Bay) (Figure A-1), identified from the otolith chemistry of juvenile Pacific herring. It was estimated that the ablated material from the edge of the adult Pacific herring otolith represents the integrated regional chemical signature of the habitat use for the three weeks prior to capture.

Appendix A continued.

Adult Pacific herring one-way ANOVA test of year class otolith signatures within bays did not find any significant ($p \leq 0.05$) differences. One-way ANOVA was restricted to adult bays and significant differences were found between the natal area signatures, nursery bay signatures, and spawning area signatures within each bay. There were also significant differences between the bays for these three adult otolith signatures. All cores from TMB and 97% of cores from SCC were inferred to have chemical signatures similar to the outer regions of PWS. When classified into the A, B, and C regional groups, all cores from TMB and 93% from SCC had signatures similar to outer PWS. No natal signatures from SCC were similar to Region A. The nursery ground signatures had diverse chemical signatures similar to all sampled regions of PWS (Table A-2).

Discussion

The adult Pacific herring results were inconclusive. The lack of similarities in the natal, nursery and spawning area signatures within and between bays indicates that these Pacific herring occupied different habitats at each life stage within and between spawning adult regions (Stransky et al. 2005) or that the regional chemistries of Pacific herring habitats have changed over time. Future studies should include broader Pacific herring sampling efforts in multiple years and in more nursery bay habitats in PWS.

Appendix A continued.

Sound inferences about spawning site fidelity cannot be made from these data. Lacking a similar reference signature between or within bays could be caused by the amount of time integrated at each life stage sampled by the laser ablation ICPMS. While juvenile Pacific herring otolith analysis integrates two weeks of habitat use, the ablated material of an adult Pacific herring otolith integrates three weeks of habitat use. The spawning adults could have an edge chemical signature which represents habitat use outside of the spawning grounds making inferences about spawning site fidelity impossible. Adult Pacific herring inhabit the coastal areas of the Gulf of Alaska ranging at an unknown scale; therefore, chemical signatures from most of the otolith material would integrate many regions, confounding results.

It has been shown that regional chemical signatures can change temporally as well as spatially (Bath et al. 2000; Gillanders 2002; Martin & Thorrold 2005). This is also the case for Pacific herring in PWS; therefore, the QDFA results are inconclusive. Lacking a complete record of the changes in the chemical signatures from the collection areas of the juvenile Pacific herring samples ending in 1997 through the collection of the adult Pacific herring samples in 2005, the QDFA results may be erroneous. Because no Pacific herring caught in 2005 were spawned prior to 2000, there is no sample overlap with individual Pacific herring. Also, because adult Pacific herring were captured in spawning areas that were not sampled for juvenile Pacific herring, there are no

Appendix A continued.

reference bays making spatial comparison inconclusive. I am unable to infer the geographic region occupied by the 2005 adult Pacific herring prior to their collection.

Future Pacific herring collection efforts should encompass multiple-consecutive years and represent many habitats utilized by Pacific herring in PWS. Because juvenile Pacific herring otoliths analysis represents a shorter period of habitat use, juvenile Pacific herring should be collected to identify regional chemical signatures in PWS. These sampling efforts should be conducted during similar season or month through multiple years. This will control for seasonal variation in chemical signatures and offer a reference 'library' of regional chemical fingerprints through time could be compared to adults by year class (Gillanders & Kingsford 2000). Adult samples should be collected from the same sites where the juveniles are collected to control for variation in signatures within regions. Adult spawning ground signatures could then be compared to the 'library' to make inferences about past habitat use in PWS and spawning site fidelity.

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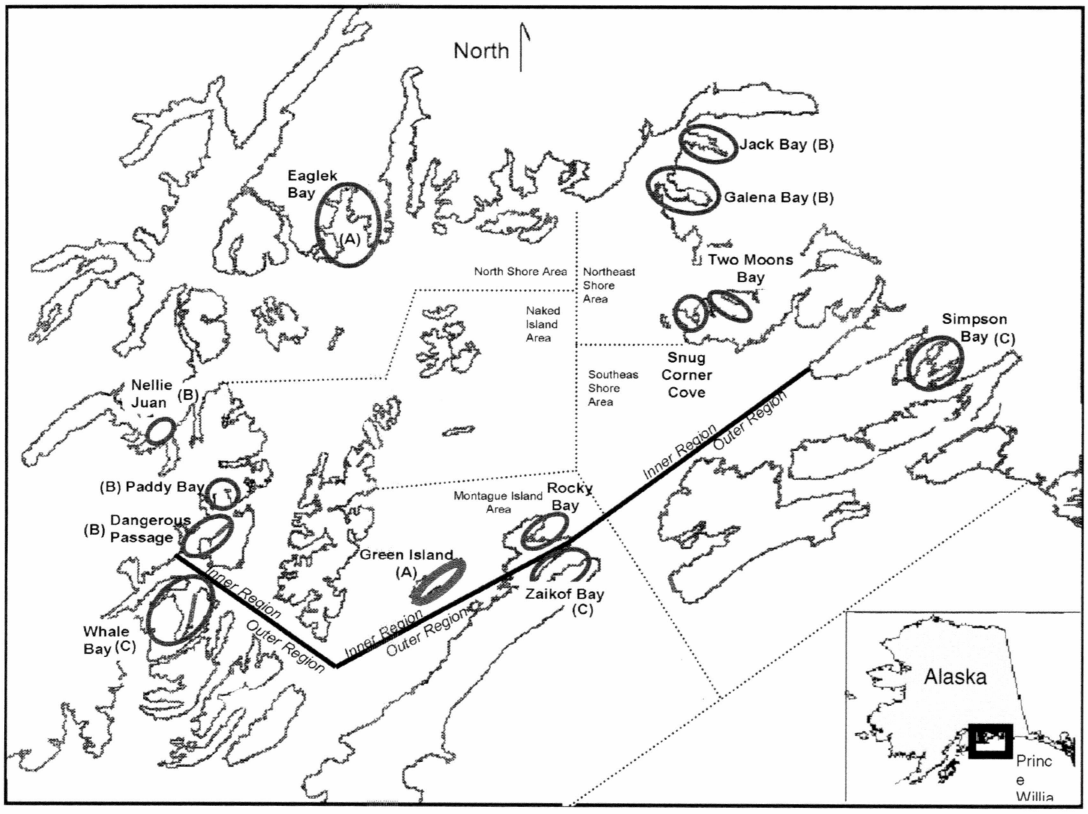


Figure A-1. Map of adult Pacific herring sampling areas in Prince William Sound Alaska. This map includes the management areas as well as the chemical regions identified by chemical analysis.

Appendix A continued.

Table A-1. Number of adult Pacific herring samples collected in Prince William Sound by capture location and age.

| Bay | Age-3 | Age-4 | Total |
|---|---|---|---|
| Two-Moons Bay | 21 | 9 | 30 |
| Snug Corner Cove | 24 | 6 | 30 |
| | | | n= 60 |

Appendix A continued.

Table A-2. Classification of adult Pacific herring otolith core chemical signatures to the regional chemical signatures of otolith edges by quadratic discriminant analysis. Regions were determined by the juvenile Pacific herring nursery signatures: (a) Inner and Outer PWS; (b) Region A= Eaglek Bay, Galena Bay, Jack Bay, Green Island Region; B= Dangerous Passage, Nellie Juan, Paddy Bay, Rocky Bay; Region C= Simpson Bay, Whale Bay, Zaikof Bay.

| | Region | | |
|---|---|---|---|
| *Nursery signature* | *Inner* | *Outer* | *Samples* |
| Two Moons Bay | 53% | 47% | 30 |
| Snug Corner Cove | 0% | 100% | 30 |
| | | | n= 60 |

(b)

| | | Region | | |
|---|---|---|---|---|
| *Nursery signature* | *A* | *B* | *C* | *Samples* |
| Two Moons Bay | 20% | 40% | 40% | 30 |
| Snug Corner Cove | 0% | 3% | 97% | 30 |
| | | | | n= 60 |

## Appendix B

Table B-1. One-way ANOVA comparing otolith edge data from juvenile Pacific herring by capture bays sampled in the same capture year. Principal components one and two are represented by PC1 and PC2. Results are indicated by the following abbreviations: df= degrees of freedom; mse = mean squared error; p = p-value.

| | 1995 Bays | | | 1996 Bays | | | 1997 Bays | | |
|---|---|---|---|---|---|---|---|---|---|
| | df | mse | p | df | mse | p | df | mse | p |
| PC1 | 6 | 4.398 | <0.001 | 8 | 5.69 | <0.001 | 3 | 12.25 | <0.001 |
| PC2 | 6 | 1.719 | <0.001 | 8 | 4.27 | <0.001 | 3 | 0.745 | 0.039 |

Appendix B continued

Table B-2. Tukey's multiple comparisons of juvenile Pacific herring otolith edge chemical signatures from bays with multiple capture years, at an $\alpha$=0.05. This table is limited to bays sampled in multiple years. Principal components one and two are represented by PC1 and PC2. Results are indicated by the following abbreviations: df= degrees of freedom.

| | Sample Years Compared | PC1 | PC2 |
|---|---|---|---|
| Eaglek Bay | | | |
| | 1995-1996 | | p<0.05 |
| | 1996-1997 | | |
| | 1995-1997 | | p<0.05 |
| Jack Bay | | | |
| | 1995-1996 | p<0.05 | p<0.05 |
| Simpson Bay | | | |
| | 1995-1996 | | |
| | 1996-1997 | | p<0.05 |
| | 1995-1997 | | p<0.05 |
| Whale Bay | | | |
| | 1995-1996 | | |
| | 1996-1997 | | p<0.05 |
| | 1995-1997 | | p<0.05 |
| Zaikof Bay | | | |
| | 1995-1996 | | p<0.05 |
| | 1996-1997 | | p<0.05 |
| | 1995-1997 | | p<0.05 |

## Appendix C

Table C-1. One-way ANOVA comparing juvenile Pacific herring edge signatures by capture month within the same capture year. Principal components one and two are represented by PC1 and PC2. Results are indicated by the following abbreviations: df= degrees of freedom; mse = mean squared error; p = p-value. Bold values indicate significance.

| Bay | | 1995 | | | 1996 | | | 1997 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | df | mse | p | df | mse | p | df | mse | p |
| Danger | PC1 | | | | 1 | 0.501 | 0.336 | | | |
| | PC2 | | | | 1 | 0.044 | 0.628 | | | |
| Eaglek | PC1 | 2 | 0.252 | 0.627 | 1 | 0.016 | 0.886 | 1 | 0.027 | 0.857 |
| | PC2 | 2 | 0.326 | 0.393 | 1 | 1.029 | 0.093 | 1 | 2.607 | **0.001** |
| Simpson | PC1 | | | | 3 | 2.714 | **0.005** | 1 | 6.507 | **0.005** |
| | PC2 | | | | 3 | 0.396 | 0.323 | 1 | 0.104 | 0.534 |
| Whale | PC1 | | | | 2 | 1.474 | 0.097 | 1 | 4.46 | **0.001** |
| | PC2 | | | | 2 | 0.492 | 0.42 | 1 | 0.003 | 0.897 |
| Zaikof | PC1 | | | | 1 | 5.302 | **0.008** | 1 | 1.617 | 0.139 |
| | PC2 | | | | 1 | 0.091 | 0.468 | 1 | 0.393 | 0.305 |

## Appendix D

Table D-1. Cross-validation test of juvenile Pacific herring otolith nursery ground signatures, using quadratic discriminant function analysis. Table shows the percentage of nursery ground otolith signatures from known geographic origins that were correctly classified by QDFA to the Pacific herring's region of capture.

| | Region A | Region B | Region C | **n** |
|---|---|---|---|---|
| Region A | 65% | 31% | 4% | 212 |
| Region B | 16% | 75% | 9% | 89 |
| Region C | 10% | 10% | 80% | 325 |
| | | | Total= | 626 |

Appendix D continued.

Table D-2 Classification of juvenile Pacific herring otolith core signatures to regional PWS chemical signatures, using quadratic discriminant analysis. Table includes all sample cores from 1995-1997.

| Region | Bay | Region A | Region B | Region C | n |
|---|---|---|---|---|---|
| | Eaglek | 21% | 65% | 14% | 121 |
| A | Galena | 5% | 85% | 10% | 20 |
| | Green Is. | 16% | 38% | 47% | 32 |
| | Jack | 3% | 49% | 49% | 39 |
| | Danger | 7% | 86% | 7% | 29 |
| B | Nellie | 0% | 42% | 58% | 26 |
| | Paddy | 12% | 82% | 6% | 17 |
| | Rocky | 18% | 77% | 6% | 17 |
| | Simpson | 3% | 75% | 22% | 101 |
| C | Whale | 0% | 18% | 82% | 110 |
| | Zaikof | 0% | 6% | 94% | 114 |
| | | | | Total= | 626 |